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The effects of remnant seed source size on plant performance in a prairie restoration

William L. Stewart
Eastern Illinois University

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THE EFFECTS OF REMNANT SEED SOURCE SIZE ON PLANT PERFORMANCE IN A PRAIRIE RESTORATION

BY

William L. Stewart

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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I HEREBY RECOMMEND THAT THIS THESIS BE ACCEPTED AS FULFILLING THIS PART OF THE GRADUATE DEGREE CITED ABOVE

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Abstract:

Tallgrass prairies have been drastically reduced to a few remnant areas that are both small and isolated. As a result, species within these areas may be exposed to a significant level of inbreeding depression and loss in genetic diversity, which can cause an overall loss of fitness in the population, increasing the chance of local extinction. In many cases, prairie restorations rely on seed collections from these small, isolated populations, which may lead to many of the same problems in the restored areas. This study specifically addressed the question: Does the dependence on seed collections from small, isolated remnants lead to loss of vigor in restored populations? To address the question, seed collections of five common prairie species were obtained from four remnant prairie populations (one large and three small). A common garden experiment was initiated in 2005, where up to 20 individuals per species per site from these populations were germinated in the greenhouse at Eastern Illinois University and transplanted into a prairie restoration site. Plants were monitored throughout the growing season for growth, vegetative reproduction, and flowering. All above ground biomass was harvested following senescence to assess total growth. Biomass varied significantly among collection sites in three of the five species (*Dalea purpurea*, *Eryngium yuccifolium*, and *Parthenium integrifolium*). However, these species did not show a consistent pattern with remnant size. Survival did not vary among collection sites for any of the species. These results suggest that plant performance is unpredictable based on remnant size and that the use of seed collections from several sites, even if a large contiguous site is available, as the best choice for restoration practice. This practice may assist restoration

efforts in avoiding the loss of genetic fitness and produce healthy, viable restored populations.

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I would like to thank Bob Faust for allowing me to use his property as a study site and allowing me to use his hydrant at no cost otherwise this project would have burnt up in the first month. Also to KSI Laboratories Inc. for analyzing soil samples free of charge. I would also like to thank everyone that helped with germination and planting. Lydia Miramontes who worked on the first half of this project as her thesis has been a big help. I had a lot of help from lab mates during the planting stage in the field: Elise Tulloss, BreAnne Nott, and Kathryn Yurkonis all volunteered their time for several days of planting. Elise has also helped a great deal with field measurements and support in general. Everyone in the lab including Scott Meiners, Elise Tulloss, Brent Wachholder, Kathryn Yurkonis, Lydia Miramontes, BreAnne Nott, Tim Rye, Jeremy Klass, and Megan Horn have all helped through their editing and revisions of various stages of proposal, abstracts, grants, and thesis. I would also like to thank all of my family and friends for their continued support in everything I do.

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Introduction:

According to Whitney (1876), prior to European colonization two thirds of Illinois was covered by grassland. The majority of the Midwest prairie that existed prior to the onset of European settlement has disappeared largely due to agricultural development throughout the area (Brothers 1990). Comparisons between existing prairie habitat and pre-European settlement conditions show that in some areas of the Midwest, including east-central Illinois, as little as 0.1 percent of the original prairie remains (Menges 1995). Of the 16 vegetation types identified in North America as having suffered the greatest decreases in size, 9 are grasslands (Menges and Dolan 1998). Given that the Midwestern U.S. has lost the majority of its tallgrass prairie habitat it makes sense that these remnant areas would also become the focus in a number of restoration efforts.

The goal of a restoration is to achieve a functional, self-sustaining community resembling one which occurred historically (Clewell et al. 2002). It has become increasingly important to use not just native species, but local genotypes of these species in restorations (Lesica and Allendorf 1999). In some instances, adaptations to local conditions or rare alleles may be lost when individuals from more distant populations are used (McKay et al. 2005). The use of seed from distant populations could therefore be detrimental to the performance of the restored population. To avoid these problems, seed used in restoration is often collected from local remnant populations. These prairie remnants are often small, isolated, and have populations with potentially lower genetic diversity due to inbreeding depression and genetic drift. The issue of source population size becomes an even more important factor when populations are under stress as small populations may show greater vulnerability to both environmental and demographic stochasticity (Oostermeijer et al. 1994). The use of seed from populations that have already been subject to some degree of loss in fitness may directly lead to many of the same problems in the restored population.

Loss of fitness in small populations

Studies have shown loss of fitness in small populations of both rare and common species resulting from inbreeding depression (Heschel and Paige 1995, Ouborg and Van Treuren 1995, Menges 1991), genetic drift (Heschel and Paige 1995, Ouborg and Van Treuren 1995, Van Treuren et al. 1991), and reduced gene flow (Jennersten 1988) though not all populations are affected (Van Treuren et al. 1993). These processes eventually decrease the genetic variation and increase the occurrence of deleterious alleles within a species (Widen 1993, Ellstrand and Elam 1993, Menges 1995). Individuals in small populations may show reduced seed size, seed viability, plant size, and increase susceptibility to environmental stress as a result of these genetic impacts.

In the predominantly out-crossed species *Gentiana pneumonanthe*, a decline in fitness occurred as a consequence of increased inbreeding within the population (Oostermeijer et al. 1994). Oostermeijer et al. (1994) showed that a lower amount of heterozygosity increased the level of variation within progeny while decreasing fitness in individuals. This suggests some maternal effects in small populations where genetically depauperate plants also give rise to poor quality offspring. This was seen in populations of *Astragalus,* where inbreeding depression increased with isolation (Karron 1989). Lower levels of germination were also seen in small populations of *Silene regia* (Menges

1991) though no effect of isolation was seen. In a study of *Ipomopsis aggregate* a decrease in plant size as well as an increase in susceptibility to environmental stress was shown. These plants showed lower levels of success in germination and a smaller seed size (Heschel and Paige 1995).

Species that have suffered a relatively recent reduction in population size may be more vulnerable to a loss in fitness than species that have persisted for a significant amount of time in a small population (Ellstrand and Elam 1993, Hauser and Loeschcke 1994). Small populations that have persisted for long periods of time may have passed through a gradual reduction in population and lost the majority of deleterious alleles that were initially present (Widen 1993). However, species that have recently declined in population size may show a much greater vulnerability to genetic drift and inbreeding depression. When population size is reduced for an extended amount of time, alleles at an individual locus have a greater chance of becoming fixed (Raijmann et al. 1994). This study also suggested that as the population size decreases, recruitment may stop, leaving only established individuals (Raijmann et al. 1994).

As our landscape becomes increasingly fragmented, more species will potentially face the dual stresses of isolation and population decrease. Decreases in mutualistic interactions such as pollination (Fischer and Matthies 1998) or environmental stochasticity (Morgan 1999, Heschel and Paige 1995, Young et al. 1996, Gordon and Rice 1998) may exacerbate these problems. In the case of drastically isolated plant species, these negative factors may increase the probability of local extinction (Kahmen and Poschlod 2000). Furthermore, these effects are anticipated for both rare and common species within communities. Therefore, it is important that the seed sources used in restoration contain a level of genetic fitness that will sustain a restored population in the long term (Gordon and Rice 1998).

Restoration implications

When designing a restoration plan, it is important to consider to what extent the area will be restored and/or what community type is desired. Restoration to pre-human disturbance is normally the target, but is rarely if ever an attainable goal due to the complexity of ever-changing ecosystems (Lockwood 1997). The end result should be a restoration of more than just the species that originally existed, but also the processes that helped to create these systems (Naveh 1994, Packard and Ross 1997). The attempt to restore habitat should also include the use of local populations, which may be difficult to obtain in areas that have been fragmented or reduced in size (Belnap 1995).

The restoration of biotic processes is linked to the overall viability of populations. Replacing processes such as disturbance and dispersal are important in allowing populations to reach self-sustaining levels (Montalvo et al. 1997). In a successful restoration populations should maintain diversity and out-crossing without supplemental planting, mowing, or other management strategies (Higgs 1997, Young et al. 2005).

Consideration should also be given to the area(s) from which seed sources are drawn to insure a large amount of genetic variation, which may increase the adaptive ability of the restored population (Knapp and Rice 1996, Gordon and Rice 1998). When additional patches and/or reintroductions are placed closely to existing populations, growth may be much faster than in areas that are restored but isolated (Huxel and Hastings 1999). This suggests that several adjacent restored patches may be required before the return of complete ecological functioning of the habitat (Huxel and Hastings 1999). Along with seed source(s), attention should be given to the landscape context of the restored surrounding areas. In areas that have been fragmented and/or isolated, corridors for pollination and dispersal may not exist, potentially reducing the success of the population (Palmer et al. 1997, Bell et al. 1997). A restoration effort using metapopulation structure can decrease isolation and aid in dispersal/movement of individuals. Gustafson et al. (2002) showed that the size of the original population of *D. purpurea* was not directly connected to genetic diversity and competitive performance. Based on their results, they recommend that restorations be conducted with multiple, local seed sources to increase the level of genetic diversity (Gustafson et al. 2002). The combination of restoring both populations and processes is critical to re-create a sustainable system from remnant areas (Packard and Ross 1997).

Project Objectives:

The goal of this project was to determine whether plant performance in a restoration is affected by the size of the seed source remnant. When locally adapted genotypes are used, seeds of prairie plants often come from small, isolated remnant populations. Data from a previous study looking at maternally affected parameters such as inflorescence, seed weight, germination rate, and seedling performance showed significant differences with respect to source population size (Miramontes, unpublished data). Reductions in at least one measure of performance were observed in all five plant species examined, suggesting that species in isolated remnants may be experiencing reduced performance which may be passed on to restoration efforts.

In my study, a common garden experiment was used to determine whether growth and reproduction within a restoration was depressed in individuals from small prairie remnants. This was conducted to look at whether relying on seed collections taken from small populations will cause a reduction in plant performance in a restoration. This hypothesis was tested using five common prairie plant species of varying breeding systems to develop generalizations that may be applied to the restoration of many systems. The goal of this research was to generate useful knowledge that will assist prairie restorationists and restoration practitioners in general. If seed collections from small remnants lead to unsustainable or weak populations, restoration methodologies should be altered to address this. If a decrease in performance is not shown, this would suggest that local seed sources could be taken from small or large remnants without the threat of decreased plant performance.

Project Methodology:

Collection and Restoration site information

Seed collection was conducted at four prairie remnants (one large, >100 ha., three small, 1 to 2 ha.) spanning the four counties of Ford, Iroquois, Shelby and Champaign. The prairie used as the large remnant runs parallel to highway 45 between the towns of Loda and Rantoul, and crosses Ford, Iroquois, and Champaign counties. This area totals approximately 260 hectares of prairie habitat along a 10.8 km section of highway. This site exists as a right of way along a railroad track and is approximately 25 m in width. Seed collections were sampled from a section near Paxton, IL that was approximately equivalent in area to the small remnants (1 to 2 ha.). This was done in order to simulate typical seed collection for restoration efforts and to avoid sampling bias in the large site.

The three small remnants consist of Loda Cemetery prairie, Paxton Cemetery prairie, and Capel Hill prairie. Loda Cemetery prairie is found in Iroquois County and consists of 1.37 ha of prairie habitat located directly adjacent to the Pine Ridge Cemetery. Agricultural fields surround the remaining edges of the site which is currently owned and managed by the Nature Conservancy. The bulk of the management of this site, as well as the other small remnants, is done through the use of periodic prescribed burns. Paxton Cemetery Prairie is located in Ford County and is currently owned by the Paxton Township Cemetery Association. This site totals 2.02 ha in area and was originally a cemetery created in 1859 and used through at least 1914 (http://www.prairienet.org/gpf/). Management practices for this area have included burns and exotic species removal. Capel Hill Prairie site is a 1.41 ha remnant along the edge of Lake Shelbyville in Shelby County (http://dnr.state.il.us/). The area is surrounded by oak hickory forest and isolated from any other prairie habitat. The IL. Department of Conservation has reintroduced fire to the site in an effort to maintain the remnant.

Plants from these collections were then used in a restoration site in Douglas County. The planting site was a 7.3 hectare plot located in Villa Grove, (Douglas County) IL. The study site was previously a crop field (the final planting in corn), and had been abandoned for one year prior to the experimental planting. Due to the short time span since abandonment, this site consisted largely of annual species and remaining corn stubble, so that removal of plants prior to the experimental planting was not necessary. The east edge of the planting site is adjacent to the Embarrass River and is slightly lower in elevation; this portion was not included in any experimental transects. The site is owned by the Villa Grove Cemetery Association and is bordered at the south edge by cemetery property currently in use. The north edge is still currently used for agriculture. The remaining edge is adjacent to the funeral home.

Soil cores were taken in the summer and fall of 2005 from all four of the seed collection sites as well as the restoration site in order to compare soil nutrient levels. Tests to determine the levels of nitrogen, phosphorus, potassium, calcium, cation exchange capacity, soil pH and percent organic matter were analyzed at KSI Laboratories in Shelbyville IL. Values from each site were averaged and soil analysis showed few differences between the prairie remnants and the restoration site (Table 1). Phosphorus was shown to be noticeably higher in the restoration site than in any of the collection sites possibly due to residual fertilizer. The restoration site was also slightly more acidic. Sites were comparable in all other aspects of soil analysis.

Species information and seed collection

All five plant species used in the experiment are commonly found in prairie habitats. Seeds were collected from; Leadplant, *Amorpha canescens* Pursh.; Purple Prairie Clover, *Dalea purpurea* Vent.; Rattlesnake Master, *Eryngium yuccifolium* Michx.; Wild Quinine, *Parthenium integrifolium* L.; and Virginia Mountain Mint, *Pychanthemum virginianum* (L.) Durand and B. D. Jackson (nomenclature follows Gleason and Cronquist 1991). These species included self-compatible species: *A. canescens, D. purpurea*, and *E. yuccifolium*; and self-incompatible species *P. integrifolium*, and *P. virginianum*, which to some degree produces seeds asexually (Molano-Flores 2004).

This variation in reproduction may help in assessing differences in performance as a result of the interaction between fragmentation and reproductive biology. Seeds were collected from up to twenty individuals of each species per site during the late summer and fall 2004. Each genotype was kept separate in a whirl pack bag during storage and stratification. For all species except *A. canescens*, which was sampled only from Highway 45 and Prospect, three sites (one large and two small) were used. The actual sample size used in the experimental planting varied between 16 and 20 individuals due to the failure of several genotypes to germinate. There were no instances were every individual of a species was sampled in any of the remnants during seed collection.

Common garden experiment

Seeds were stratified in moist perlite for a three-month period at 5° C. Two species, *Amorpha canescens* and *Dalea purpurea*, require scarification before stratification, which was done by scraping the seed with a fine grit sand paper to remove a portion of the seed coat. Seeds from each genotype were germinated in Petri dishes lined with moist filter paper and placed in a growth chamber at 20° C. Seedlings were then transplanted into 164 ml individual conetainers (Stuewe & Sons, Inc., Corvallis, OR) in a sterile potting mix (Pro-Mix, Premier Horticulture Inc., Quakertown, PA) and grown in the Eastern Illinois University greenhouse. All individuals were moved outside of the greenhouse to harden off for a period of three to four weeks prior to transplanting into the field site.

Seedlings were transferred to the restoration site in the spring of 2005. The experimental planting was a 12 by 220 meter portion of the Villa Grove site, which contained four transects with 2 meter spacing between rows. Each seedling was marked with both a flag and a metal tag. Individuals were planted randomly, by species and source population, along transects with 2 meter spacing to allow for growth and eventual clonal expansion. Additional watering was used on two occasions as needed during drought conditions early in the summer of 2005. Replanting of dead individuals was done in mid-July and early-November but these individuals were excluded from survival analyses.

Plants were monitored throughout the growing season for growth, vegetative reproduction, and flowering. Surveys were also conducted to assess possible insect damage, however no significant insect herbivory was recorded. Height measurements were taken from 23 June through 9 Sept. Following this period, only mortality was monitored as the plants had begun to senesce. Following the senescence of each plant, all aboveground biomass was harvested and dried to determine total growth as a measure of plant performance and vigor. In the spring of 2006, over-winter survival was determined for all remaining transplants.

One-way ANOVA's were used to assess the effect of source population on total biomass of each species. Variation among species within each individual collection site were also assessed using ANOVA tests. This was incorporated to look at possible variation due to reduction in habitat size between species sampled from a single site. *Dalea purpurea* samples from Capel Hill prairie were not included in these analyses because no other species were sampled from that site. Log transformation was used in all biomass analyses to comply with ANOVA assumptions. Total growth rate was calculated using a 57 day portion of plant height data from 14 July to 9 September. This time period began one month after the initial planting and was selected because it contained a window of plant growth after transplant shock that ended prior to senescence. This was used to assess possible differences in plant growth within species among collection sites. A survival analysis using Cox regression was used to assess variation in performance among collection sites.

Survival analysis using Cox regression was also used to assess differences among species. *Pycanthemum virginianum* showed the lowest risk of mortality and had the highest final sample size. Therefore it was used as the comparison species in the survival analysis. All data analyses were conducted using SPSS version 13.0 (SPSS Inc. Chicago, IL). Significance for all statistical analyses was set at $P<.05$.

Herbivory Experiment

A greenhouse herbivory experiment was conducted to assess the variation in stress tolerance between individuals taken from small and large remnants. Two species were used in this experiment, Leadplant, *Amorpha canescens* and Virginia Mountain Mint, *Pychanthemum virginianum*. Seeds were germinated in Petri dishes with moistened filter paper and placed in a germination chamber. Scarification was again used on *A. canescens* seeds using a fine grain sand paper to remove a portion of the seed coat. Seedlings from the germination chamber were transplanted into individual 2.5 x 2.5 x 2.25 cm cells and transferred to the EIU greenhouse. Two individuals from each genotype were used so that one individual could be manipulated and one kept as an experimental control.

Seedlings were grown for a 4 week period prior to the manipulation. Herbivory was simulated by clipping and removing the top 50% of one plant of each genotype. A percentage was used instead of a set length in order to keep the relative damage to each plant equivalent. Plant growth was monitored for an additional 37-day period prior to harvest. Harvested biomass was oven dried at 63° C and weighed. Plant height was also monitored over the course of the entire experiment to assess the level of re-growth following simulated herbivory.

Final growth measurements were converted to percent loss relative to control (100% * control-clipped/control). This analysis was used to look at changes in growth between the control and the stressed individuals while controlling for genotypic variation. Percent loss was arc sine transformed and log transformation was used for analysis of height data. ANOVA was used to determine differences in overall herbivory response among sites.

Table 1. Soil analysis results from four seed sample sites and restoration site. Soil analysis conducted by KSI laboratories, Shelbyville, IL.

	Loda (small)	HW 45 (large)	Prospect (small)	Capel Hill (small)	Villa Grove (planting site)
PPM Nitrate	1	1	1	1.5	1.5
Soil pH	6.8	7.2	6.9	7.4	5.8
Buffer pH	7	7	7	7	6.5
P kg/ha	17.9	11.7	15.15	9.7	70.6
K kg/ha	535.5	453	405.5	261.6	343.6
Ca kg/ha	5715	5550	5660	4880	2923.3
Mg kg/ha	1210	1340	1310	768.6	477.3
% Org. mat.	3.5	3.5	3.6	2.6	3
Cation exchange capacity	18.6	17.8	18.3	-2.9	11.4
% Ca	68.6	69.4	69	42.6	57
% Mg	24.2	28	26.6	9.6	15.6
% K	3.3	2.9	2.6	1.2	3.5
% H	3.8	not tested	1.7	not tested	23.8

Results:

Species Performance in Restoration

Species varied dramatically in their performance within the restoration site. There were significant differences in biomass across species at Highway 45 ($F_{4,59}$=10.845, P=<0.001, R^2=0.424), Loda Prairie ($F_{2,44}$=7.086, P=0.002, R^2=0.244), and Prospect Prairie ($F_{4,65}$=11.503, P=<0.001, R^2=0.414) (Figure 1 a-c). Plant performance across a single source site was generally consistent across all five species. *Pycanthemum virginianum* showed the highest performance level in biomass among the five species at Highway 45 and Loda. *Parthenium integrifolium* also did well across all sites. *Eryngium yuccifolium* had relatively consistent biomass across all three of the sample sites (Figure 1 a-c). *Dalea purpurea* showed the lowest performance levels in both the Highway 45 and Prospect sites. *Parthenium integrifolium* showed the lowest performance across species for the Loda site, although *D. purpurea* and *A. canescens* were not found at this site. *Parthenium integrifolium* showed the highest performance in biomass at Prospect Prairie. Post hoc analysis of the site showed *P. virginianum*, *E. yuccifolium*, and *P. integrifolium* in the larger of the two groups.

Survivorship also varied among species with *D. purpurea* and *E. yuccifolium* having the greatest relative risk of mortality (Figure 2 a). The highest survivorship was seen in *P. virginianum,* which was used as the reference species for relative risk assessment. Significant variation in survivorship among species was seen in the Cox regression (χ^2 =50.458, df=4, P<0.001). Within the survival analysis, individual contrasts with the reference species were significant only for *E. yuccifolium* (χ^2=24.400, df=1, P<0.001) and *P. integrifolium* (χ^2=12.556, df=1, P<0.001).

Influences of Remnant Size on Plant Performance

Biomass data - This experiment showed variation among sites in overall plant performance. Analysis showed significant variation in final biomass among remnants in three of the five species tested (Figure 3). Significance was seen in *D. purpurea* ($F_{2,21}$=3.485, P=0.049, r^2=0.249), *E. yuccifolium* ($F_{2,37}$=3.263, P=0.049, r^2=0.150), and *P. integrifolium* ($F_{2,43}$=4.266, P=0.020, r^2=0.166). Significant differences in *D. purpurea* were seen between the two small sites but not with the large site (Highway 45), which was intermediate in biomass. *Eryngium yuccifolium* biomass was significantly greater in the Loda site (small) than in both the Highway 45 (large) and Prospect (small) sites. Finally, *Parthenium integrifolium* biomass was significantly smaller in the Highway 45 site than in Prospect prairie. This was the only species that showed a significant pairwise difference between one of the small sites and the contiguous Highway 45 site. However, this was opposite to the original hypothesis in that the larger remnant had the lower biomass. Overall, differences in biomass data among sites did not consistently support the original hypothesis that the size of the source remnant would be associated with reduced plant performance.

Growth rate data – Significant difference in height growth among sites was found for only one of the five species. *Pycanthemum virginianum* had significantly higher growth rate in the Prospect site ($F_{2,45}$=3.749, P=0.031, r^2=0.143) (Figure 4). However, no difference was seen between Highway 45 and Loda for *P. virginianum*. The other four species showed no variation among sites (P>0.945). The Highway 45 population of *D. purpurea* and the Prospect population of *A. canescens* both had negative height growth due to drought associated die back and mammal damage. The combination of these factors increased the variation in the data set shown by the large standard error in all species except *P. virginianum*. *Pycanthemum virginianum* had the least amount of overall damage in comparison to the remaining four species.

Survival analysis – Survivorship overall was relatively high across collection sites and species. Cox regression revealed no significant effects of collection site on survivorship for any of the five species tested individually. Therefore, there was no influence of remnant size on survival (χ^2=0.154, df=2, P<0.926).

Reactions to Simulated Herbivory

Herbivory data showed only weak support for a difference between small and large populations. There was no effect of collection site on total final biomass for *A. canescens* ($F_{1,32}$=1.944, P=0.173, r^2=0.057) and *P. virginianum* ($F_{2,36}$=0.223, P=0.802, r^2=0.012) (Figure 5). Re-growth was seen in both species as well as additional vegetative shoots in *P. virginianum*. Again weak support was shown for an effect of remnant size on performance with significant differences observed in a comparison between average height of control and manipulated plants (Figure 5). Analysis of variance on final height data showed a significant site effect for *A. canescens* ($F_{1,33}$=5.142, P=0.030, r^2=0.135) but not for *P. virginianum* ($F_{2,38}$=0.837, P=0.441, r^2=0.042) (Figure 5). Significant differences in A. *canescens* height were seen between Highway 45 and Prospect (Figure 5). These data show weak support for differences in plant performance between sites, with *A. canescens* showing an increased ability to recover in height in the Highway 45 population.

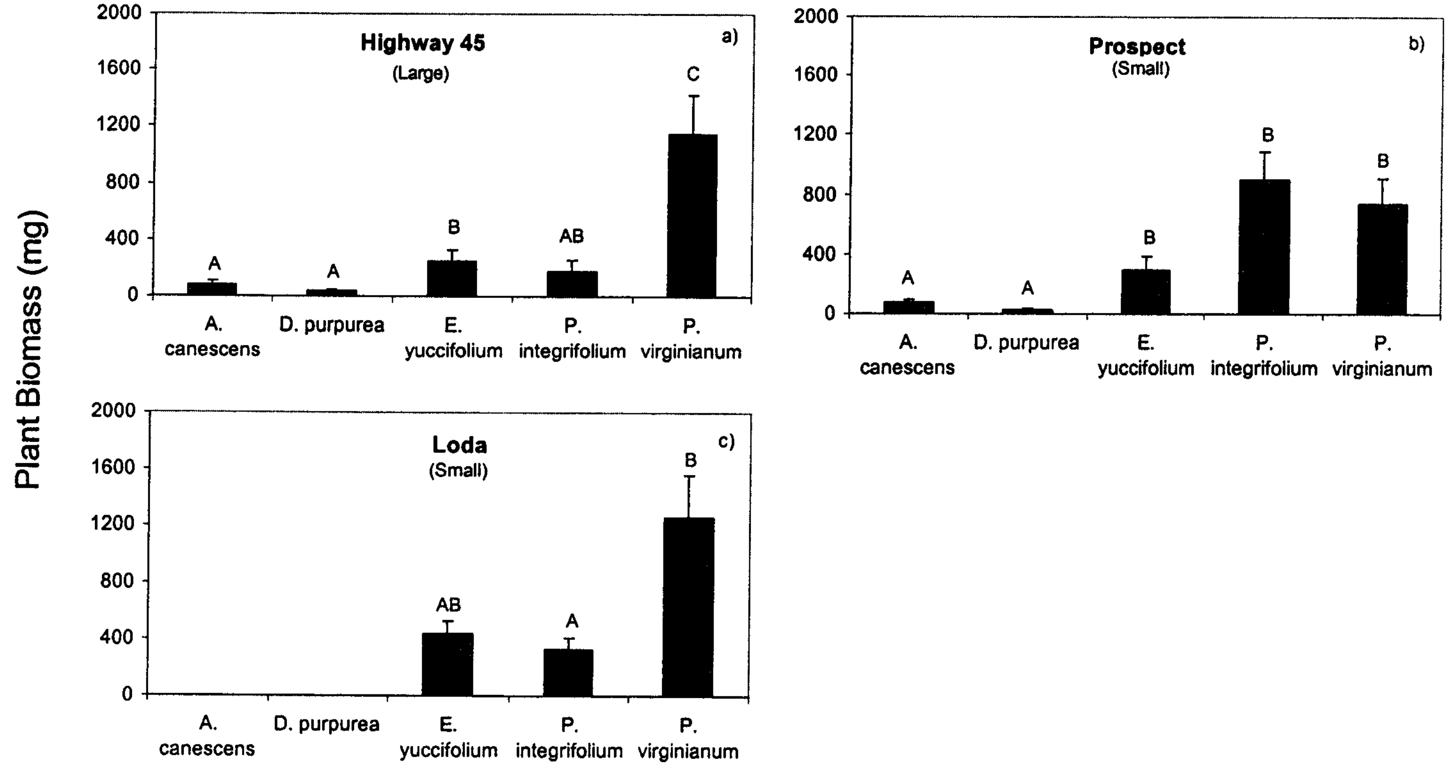

Figure 1. (a-c) Biomass of all species within a single sample site. Data plotted are a) Biomass across species at Highway 45 sample site (mean ± SE) b) Biomass across species at Prospect sample site (mean ± SE) c) Biomass across species at Loda sample site (mean ± SE). Letters above std. error bars show significant differences between species. Means with the same letter within a site are not significantly different based on a Duncan post hoc test.

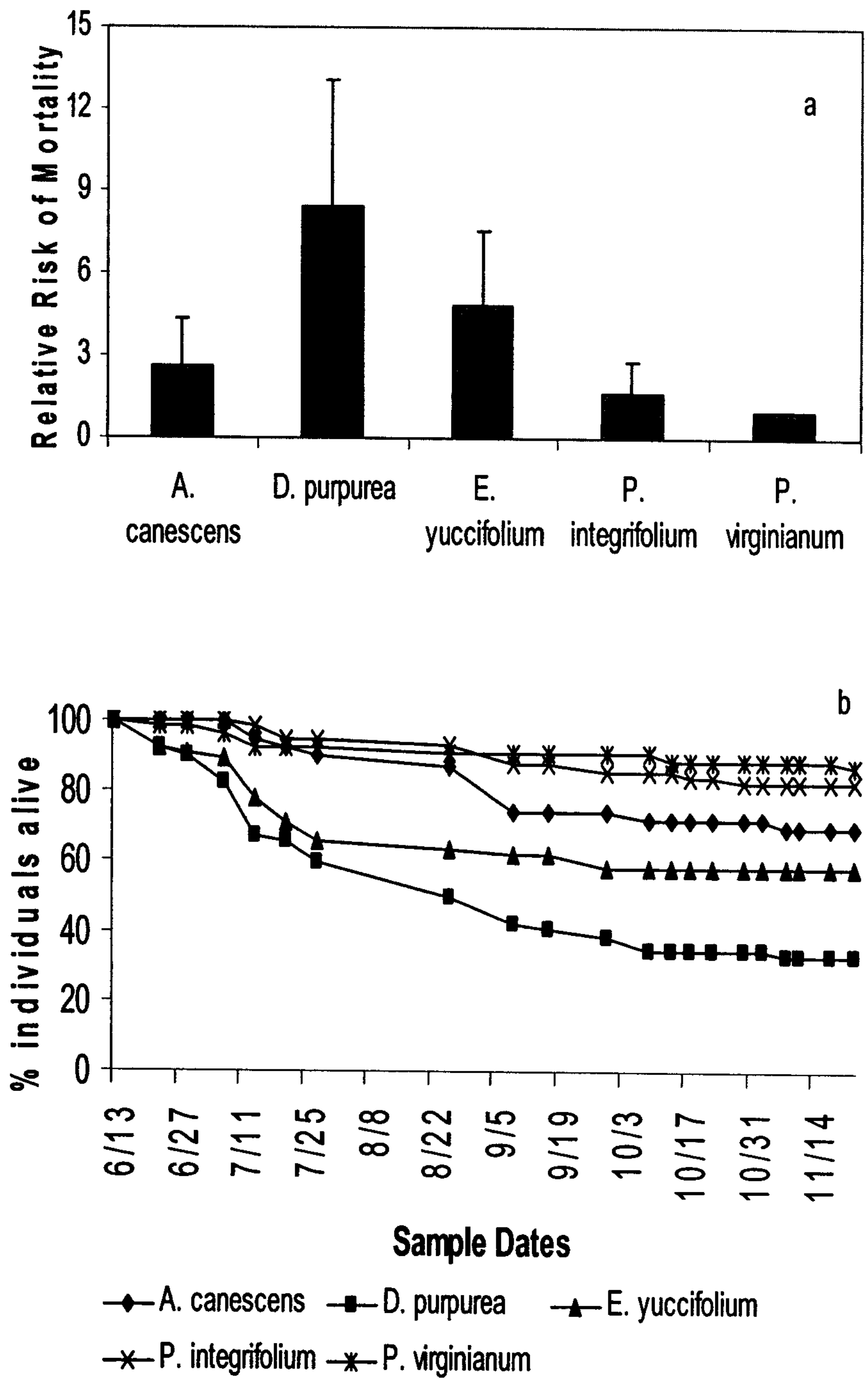

Figure 2. (a-b) Mortality of five prairie species, all sites combined, planted at the restoration site. Data plotted are a) Relative risk of mortality estimated for a Cox regression (Mean ± SE) and b) Survivorship curves of all planted seedlings.

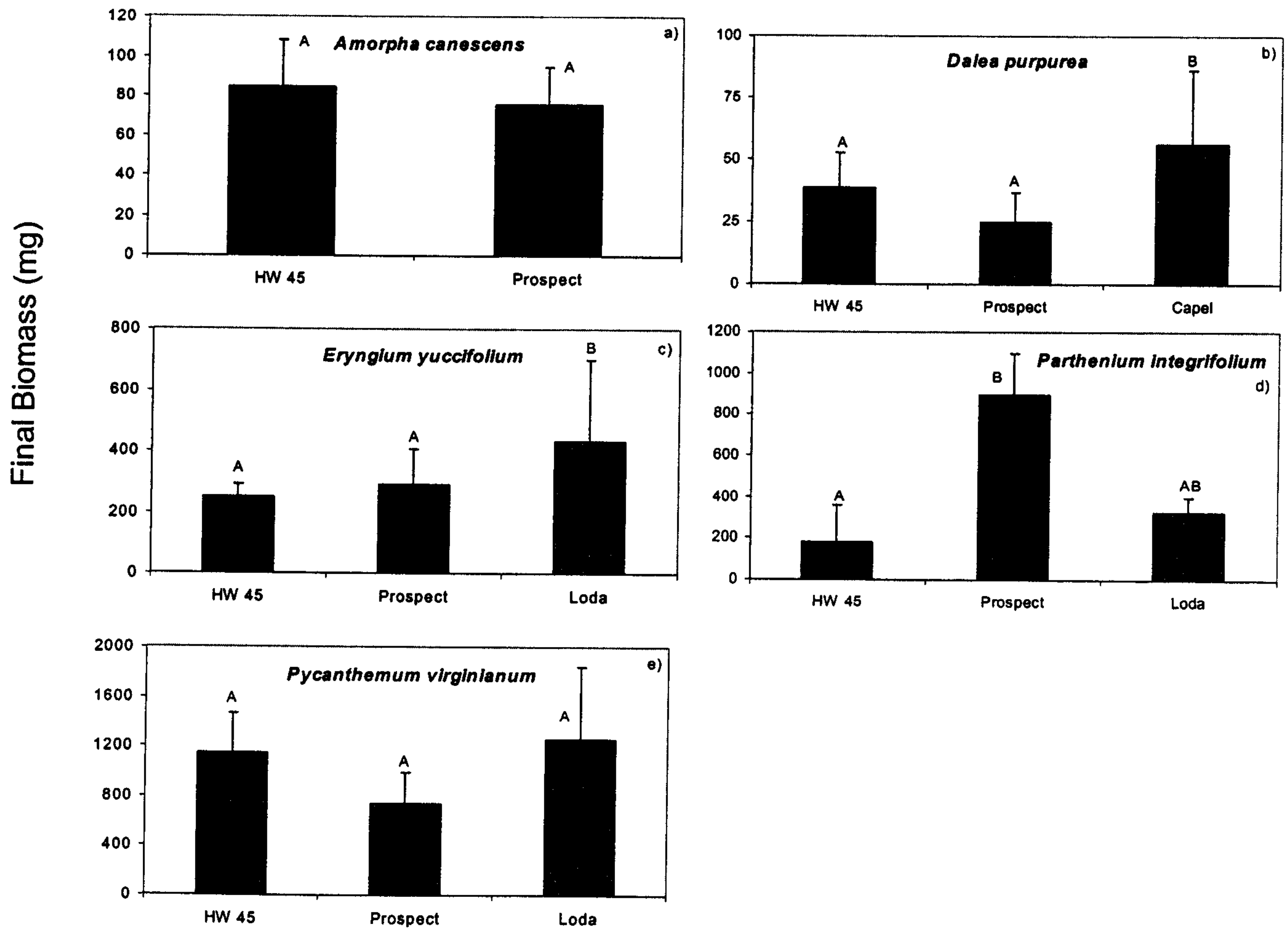

Figure 3. (a-e) Biomass of species across sample sites. Data plotted are biomass (mean ± SE) of a) *Amorpha canescens* b) *Dalea purpurea* c) *Eryngium yuccafolium* d) *Parthenium integrifolium* e) *Pycanthemum virginianum*. Letters above std. error bars show significant differences between sites. Means with the same letter within a species are not significantly different based on a Duncan post hoc test.

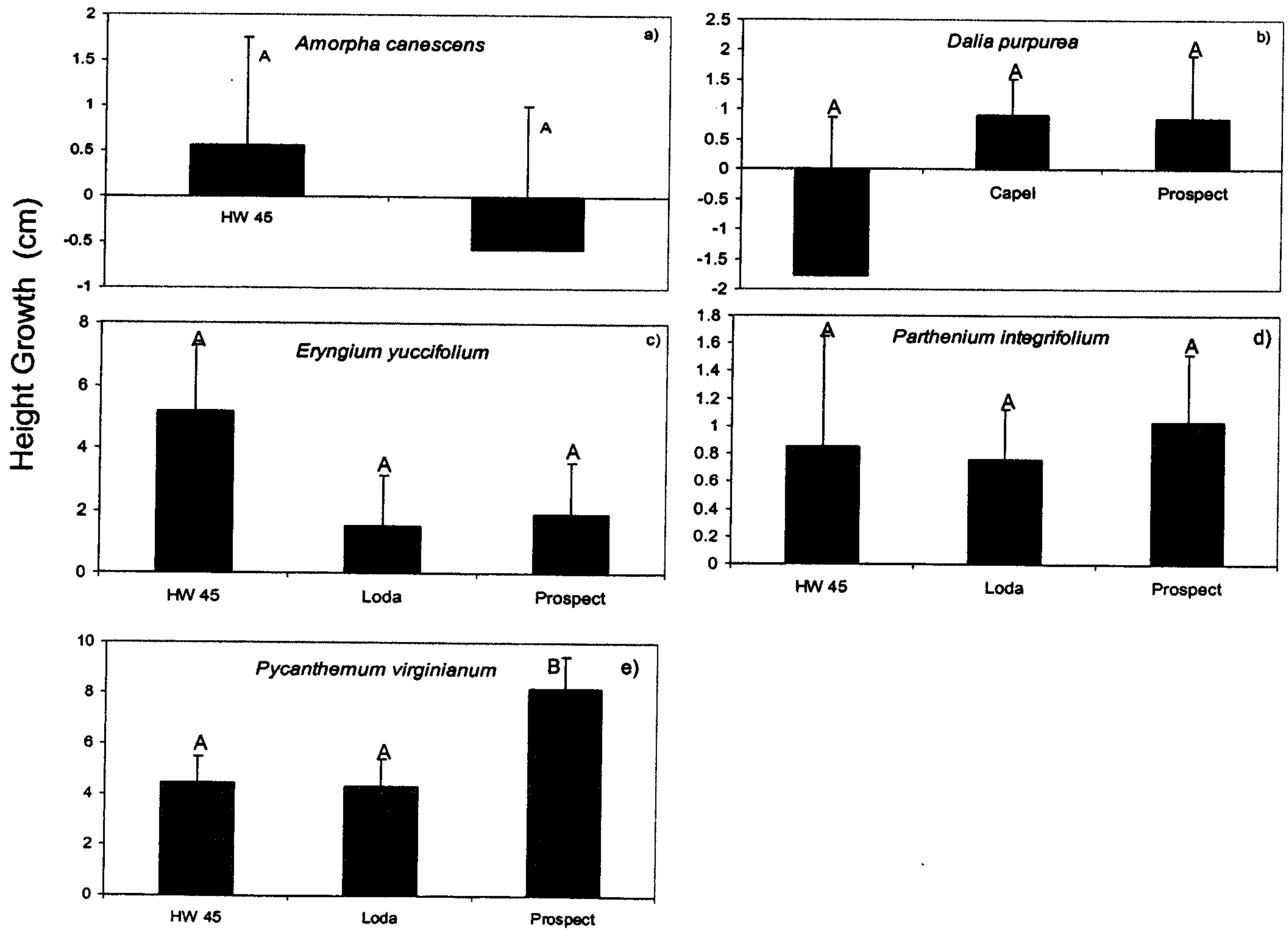

Figure 4. (a-e) Average growth rate of individual species across sample sites. Data plotted are heights (mean ± SE) a for a) *Amorpha canescens* b) *Dalea purpurea* c) *Eryngium yuccifolium* d) *Parthenium integrifolium* e) *Pycanthemum virginianum*. Letters above std. error bars show significant differences between sites. Means with the same letter within a species are not significantly different based on a Duncan post hoc test.

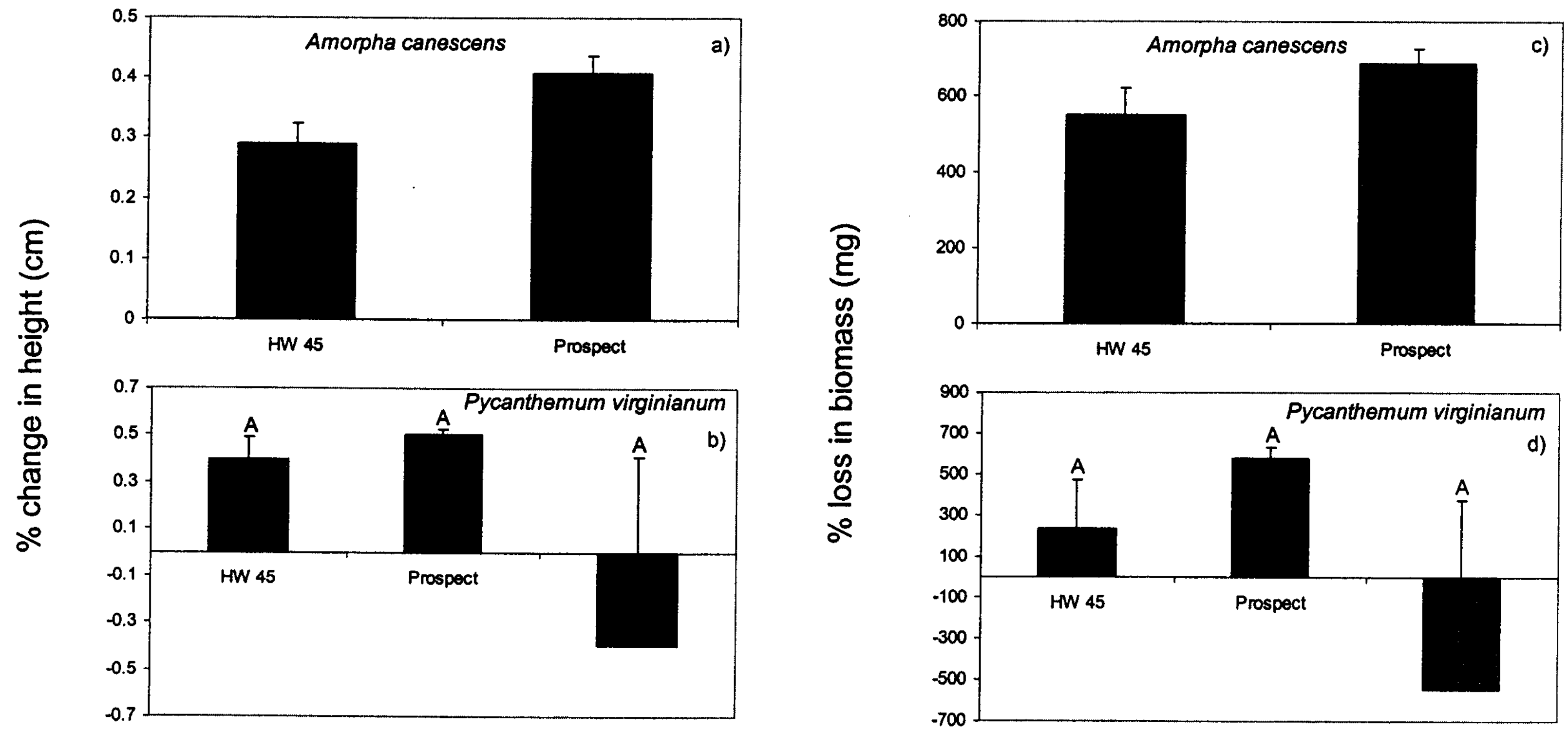

Figure 5. (a-d) Percent change in height and percent loss in biomass calculated as control-treatment/control. Data plotted from herbivory simulation are a) Average percent change in height for *Amorpha canescens* (mean ± SE) b) Average percent change in height for *Pycanthemum virginianum* (mean ± SE) c) Average percent loss in biomass for *Amorpha canescens* (mean ± SE) d) Average percent loss in biomass for *Pycanthemum virginianum* (mean ± SE). Letters above std. error bars show significant differences between sites. Means with the same letter within a species are not significantly different based on a Duncan post hoc test.

Discussion:

Species Performance Across Sites in a Restoration

Biomass data were analyzed to look at variation among species within a single sample site. Significant differences among species within a single site were seen in three of the sample populations. These results were generally consistent across all five species. In both Highway 45 and Loda, *P. virginianum* had the highest biomass levels and was second to *P. integrifolium* at the Prospect site. *Dalea purpurea* and *A. canescens* had the lowest biomass at both Highway 45 and Prospect. Low performance of *D. purpurea* and *A. canescens* can be attributed in part to loss of individuals from deer browse and transplant shock. Transplant shock in this case may have been increased due to drought conditions early in the growing season. *Parthenium integrifolium* had the lowest final biomass for the Loda site. This species showed the highest level of variation in biomass among collection sites. The greatest amount of biomass was seen in HW 45 and Prospect and reduced biomass at Loda. *Eryngium yuccifolium* was generally constant across the three collection sites. Biomass levels in *E. yuccifolium* were significantly lower than *P. virginianum* and *P. integrifolium* in most cases which may be due in part to a small degree of plant damage from burrowing mammals.

Survivorship overall was also shown to vary significantly among species. The highest level of survivorship was seen in *P. virginianum* and the greatest risk of mortality in *D. purpurea* and *E. yuccifolium*. This increased risk may be attributed in part to greater loss in individuals of *D. purpurea* from herbivory and transplant shock. This species appears to have a much greater difficulty initially becoming established. Small remnant populations of *D. purpurea* were shown to retain a large amount of genetic diversity when seed samples were used in a restoration (Gustafson et al. 2002) which suggests that a loss in genetic diversity was not driving poor plant performance.

Parthenium integrifolium was relatively consistent in survivorship among sites although significantly lower than the reference species (*P. virginianum*). *Eryngium yuccifolium* had much greater mortality in comparison to *P. virginianum* most of which occurred early in the season (Figure 1b). *Eryngium yuccifolium* may exhibit weedy species characteristics in some instances within a restoration effort (Molano-Flores 2001, Cottam and Wilson 1966). However, populations of *E. yuccifolium* are naturally controlled in many cases by the larva of a moth species (*Aristotellia* sp.) (Molano-Flores 2001). Due to *E. yuccifolium* being documented as one of the most common species in prairie seed banks (Johnson and Anderson 1986), the absence of this herbivore could allow for a large increase in numbers despite its initially low survival (Molano-Flores 2001).

Influences of Remnant Size on Plant Performance

Biomass data - No evidence was found for a difference in plant performance in relation to remnant size. It is often expected that populations that have decreased in size and become isolated will also suffer decreases in heterozygosity (Kolb 2005). This decrease may cause a loss in fitness attributed to inbreeding depression and genetic drift (Heschel and Paige 1995, Ouborg and Van Treuren 1995), and may lower the ability of a population to survive under adverse environmental conditions (Kolb and Lindhorst 2006). However, it has also been hypothesized that if given a large enough span of time all deleterious alleles may be purged from a small population (Widen 1993, Barrett and

Charlesworth 1991). This does not appear to clearly be the case in this study. Historic land purchase data shows that the sample areas first became fragmented between 141 and 171 years ago. Given that these are all perennial species, this may be a long enough period to discard deleterious alleles, but due to the level of variation in plant performance among remnants this may not have occurred for all species.

A significant effect of remnant identity was seen in three of the five species, but these differences were not always found to be between the small and large remnants. Significant among-site differences in *D. purpurea* were found between Prospect Cemetery and Capel Hill Prairie, both of which are small remnants but there was no difference with Highway 45. *Eryngium yuccifolium* results showed that a significant difference exists between Loda Cemetery Prairie and both Highway 45 and Prospect Cemetery Prairie as a single group. Again this shows a difference between small and large sites, but a small remnant had the greater biomass. Similarly, analysis of *P. integrifolium* showed differences between the Highway 45 site and the Prospect site, with the smaller site displaying better performance, and no difference in respect to Loda Cemetery Prairie. The poor performance of some species in the large site suggests that these species may have been effectively fragmented by the narrow nature of the site and are now exhibiting a loss in vigor.

These results are in disagreement with several previous studies conducted showing support for a correlation between decreased fitness and small population size (Oostermeijer et al. 1998, Heschel and Paige 1995, Eisto et al. 2000, Mavraganis and Eckert 2001). While variation was seen among the fragmented prairie remnants it may be that some of the small remnants still have a population that is sufficient to not show significant reduction in performance. A lack of reduction in fitness in small populations and the failure to see a significant effect of genetic erosion on fitness has been shown in a study of *S. pratensis* (Ouborg and Van Treuren 1995, Van Treuren 1993, Hauser and Loeschcke 1994) this suggests that some small remnants may have purged deleterious alleles from the gene pool or may still maintain a large enough population to avoid negative effects of inbreeding depression and genetic drift. Alternatively the fragmentation may have been too recent for these perennial species to show a reduction in performance.

This lack of relationship between reduction in habitat and reproductive output has been documented previously in a study of reproductive success among forest herbs (Bruna and Kress 2002, Kolb and Lindhorst, In Press). These results also conflict with the preliminary data that showed significant differences among populations with collections from the large remnant often performing better (Miramontes unpublished data). The experimental design of this field study largely excluded the maternal effects examined in the preliminary data, as germination and initial seedling growth took place under greenhouse conditions. However, since differences among populations were also observed during growth trials in the greenhouse (Miramontes unpublished data), these same differences were expected in the common garden experiment but were not found. Results from this study suggest that rearing individuals in the greenhouse and transplanting as seedlings may have helped to overcome a barrier to performance that may have otherwise been seen if seed had been directly planted into the restoration site. Flowering did not occur in the first season of growth, which will be the dominant factor in the ultimate success of the restored populations.

The lack of consistent seed source effects may be affected by variation in the data set created by random herbivory damage and transplant shock brought on by drought conditions. Supplemental watering was used on two occasions in an attempt to minimize drought effects though plants were still visibly stressed. Herbivory damage was seen mainly in *Amorpha canescens* and *Dalea purpurea* and was attributed mainly to deer browse. Some damage was also seen in *Eryngium yuccifolium* due to small mammals burrowing. This damage was not directly associated with the plant but with the loose soil around each transplant. Herbivore damage may be responsible in part for the relatively poor model fit seen in the analyses.

Data from a previous study using the same seed samples showed support for the decreases in seed set, seed weight, and germination often seen in small populations (Eisto et al. 2000). A decrease in seed weight was shown in a study of *Salvia pratensis* showing support for a significant level of inbreeding depression (Ouborg and Van Treuren 1995). This was also seen in a study of *Gentiana pneumonanthe* that used several fitness components that were correlated with population size showing a reduction in fitness (Oostermeijer et al. 1994). A decrease in reproduction of two out-crossed species was also seen in a study of both *Sanicula* and *Phyteuma* (Kolb 2005, Kolb and Lindhorst 2006). Three of the species used in this study (*A. canescens, D. purpurea*, and *E. yuccifolium*) are also self-compatible which is where genetic problems would be expected to develop (Eisto et al. 2000). These reproductive issues would become evident as the restoration planting matured.

Growth rate and Survival analysis – Growth rate data showed significant difference among sites only for *P. virginianum*. However this was seen only for Prospect

Prairie, which grew faster than the Highway 45 and Loda Cemetery Prairie genotypes, opposite the hypothesized direction. Survival did not vary across sites for any species. This could be attributed to loss of individuals from herbivory as the main source of mortality and that all plants were well established when planted.

Reactions to Simulated Herbivory

Analysis of height and biomass data for the greenhouse herbivory experiment showed a weak effect of remnant size on performance. Final biomass and growth rate in control and manipulated plants both showed no effect of remnant size on plant performance of *P. virginianum*. However, *Amorpha canescens* showed significantly better re-growth in the large remnant. Based on the data from a previous study using the same seed samples, which showed a large influence of remnant size on plant performance in the greenhouse growth, a larger effect was expected (Miramontes unpublished). However, in this herbivory experiment a reduction in biomass as an effect of collection site was not found for *A. canescens*. The lack of response may be attributed to the slow growth of *A. canescens* in comparison to *P. virginianum*. This result is in agreement with a study by Heschel and Paige (1995) which showed that increased susceptibility to environmental stress, using simulated herbivory, was seen in small populations of *Ipomopsis aggregate*.

Conclusions:

This study may be important in guiding management decisions in restoration efforts. In general, no consistent effect of remnant size on plant performance was seen, suggesting that seed collections could be taken from small native populations without concern of a loss in performance. However, plant performance across all remnants was quite variable. Since plant performance varied unpredictably across the source population and species, it may be likely that inferior genotypes could be introduced into restorations even if collected from large remnants. In order to ensure that performance is maintained, it may be advantageous to use collections from multiple seed sources.

Although multiple seed sources may alleviate inbreeding effects, it may also introduce problems from outbreeding depression. However, a study looking at both inbreeding and outbreeding depression of varying distances found that F1 generations out-competed the parents, and F3 generations were usually of a comparable or greater fitness level to the parent (Fenster and Galloway 2000). This study suggested that outbreeding depression may not be a significant problem in a restoration effort as long as the seed source populations are not in excess of 100 km apart. These areas would also need to be within this 100 km proximity to the restoration site to preserve localized adaptations (Fenster and Galloway 2000, Galloway and Fenster 2000, McKay et al. 2005). The lack of outbreeding depression supports the utility of mixing several source populations within a restoration (Fenster and Galloway 2000, Gustafson et al. 2002, McKay et al. 2005).

My results should be applicable to a large number of systems since the species used were common, while most of the studies in this field focus on rare or endangered species. This suggests that even common species may exhibit reduced performance within small, isolated remnants. Overall my results suggest that seed collections from multiple local source populations will be a viable method of ensuring population viability within restorations. Clearly, there is large variation among species performance in fragmented populations. More long-term studies are needed to assess the extent of this variation and to determine its impact on restorations.

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